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(57) Abstract

The immune response to a DNA immunogen in a mammal can be enhanced by administration of a chemokine or a polynucleotide encoding the chemokine. This method can be used, for example, to immunize or vaccinate a mammal against an infectious disease or a tumor.

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ENHANCING IMMUNE RESPONSES TO GENETIC IMMUNIZATION

This application claims the benefit of co-pending provisional application Serial No. 60/082,600 filed April 22, 1998, which is incorporated by reference herein.

TECHNICAL AREA OF THE INVENTION

The invention relates to the area of immune responses to genetic immunization. More particularly, the invention relates to enhancing immune responses to DNA immunogens using immune co-stimulatory molecules.

BACKGROUND OF THE INVENTION

The use of genetic immunization, or immunization with DNA encoding polypeptide immunogens, to prime immune responses is viewed as a promising vaccine strategy. This technology offers potential improvements compared to other types of vaccines, such as subunit proteins complexed with adjuvants or inactivated or attenuated viral preparations. In addition to the practical advantages of simplicity of construction and modification, injection of genetic material encoding for polypeptide immunogens results in synthesis of the immunogens in the host. Thus, these immunogens are presented to the host immune system with native post-translational modifications, structure, and conformation.

In mice, several DNA vaccines have been effective at inducing long-lived antibody and cytotoxic T lymphocyte (CTL) responses and have conferred protective immunity against a number of viruses, bacteria, parasites, and tumors (1-8). Various

approaches to enhance immune responses mediated by genetic immunization have been investigated. In addition to variations in dosage, route or boosting regimens, these variations include co-injection of polynucleotides encoding co-stimulatory molecules which improve immunogen presentation to lymphocytes, such as B7-1 or B7-2, or cytokines, such as GM-CSF, IL-2, IL-2, and IL-12, to create an optimal cytokine microenvironment for T cell priming (11-19). However, further enhancement of immune responses to genetic immunization is desirable for immunizing mammals, particularly humans, against immunogens such as virus- and tumor-specific immunogens.

Thus, there is a need in the art for methods of enhancing the immune responses to DNA immunogens.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a method of enhancing an immune response to a DNA immunogen. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention provides an immunogenic composition. The composition comprises a DNA immunogen and a chemokine or a polynucleotide encoding a chemokine.

Another embodiment of the invention provides a method of enhancing an immune response to a DNA immunogen in a mammal. A chemokine or a first polynucleotide encoding a chemokine and a DNA immunogen are administered to the mammal. An immune response to the DNA immunogen is thereby enhanced.

The present invention thus provides the art with the information that chemokines can be used to enhance an immune response of a mammal to a DNA immunogen. The invention can be used to, *inter alia*, to immunize or vaccinate a mammal against an infectious disease or a tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Figure 1 shows the immunization and bleeding schedules for animals immunized with HCV immunogens.

Figure 2. Figure 2 shows the immunization and bleeding schedules for animals immunized with granulocyte-macrophage colony-stimulating factor (GM-CSF).

Figure 3. Figure 3 shows the immunization and bleeding schedules for animals immunized with HCV immunogens and RANTES.

Figure 4. Figure 4 shows the immunization and bleeding schedules for animals immunized with HCV immunogens and macrophage inflammatory protein 1α (MIP-1α).

Figure 5. Figure 5 shows the increased anti-HIV gag antibody titer in mice immunized with a plasmid encoding HIV gag and a plasmid encoding the chemokine B lymphocyte chemokine (BLC).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is a discovery of the present invention that administration of a chemokine or a polynucleotide encoding a chemokine can be used to enhance an immune response in a mammal to a DNA immunogen. This method can be used, *inter alia*, to increase immunological resistance to pathogens, such as viruses and bacteria, and to tumor-associated immunogens.

Chemokines generally function as chemoattractants for cells which they recruit from the blood to sites of infection. Thus, administration of a chemokine, either together with or in addition to a DNA immunogen, effectively recruits various cell populations, including antigen presenting cells and effector cells, to the site of administration or its vicinity. Similarly, administration of a polynucleotide encoding a chemokine can result in local chemokine secretion which induces migration of antigen presenting cells and/or lymphocytes to the site of administration and which enhances immune responses to the DNA immunogen. Local chemokine secretion can also enhance the migration of cells which have taken up the DNA immunogen or

polypeptides encoded by the DNA immunogen to the lymph nodes, where priming of specific T cells can occur.

Chemokines which can be used in the method of the invention include, but are not limited to, B lymphocyte chemokine (BLC), IL-8, PBP/β-TG/NAP-2, macrophage inflammatory proteins MIP-1α, MIP-1β, and MIP-3α, macrophage chemoattractant and activating factor (MCP-1 or MCAF), MCP-2, MCP-3, I-309, C10, HCC-1, RANTES (regulated upon activation, normal T cell expressed and secreted), lymphotactin, SCM-1, eotaxin, MGSA, PF4, NAP-2, IP-10, ENA-78, EMF-1, GCP-2, SLC, ELC, and SDF-1. Certain chemokines may be more effective in combination with a particular DNA immunogen than others at stimulating an immune response; optimization of the DNA immunogen-chemokine combination can be carried out using routine assays in standard animal models (see Examples 1 and 2).

The immune response which is enhanced can be any response which is influenced by chemokines, including, but not limited to, antibody production or cytotoxic T lymphocyte (CTL) response resulting from chemoattraction and/or activation of antigen presenting cells, such as dendritic cells, macrophages, and monocytes, chemoattraction and/or activation of neutrophils, including eosinophils, and chemoattraction and/or activation of naive T cells, memory T cells, and pre-T cells to the thymus.

Measurement of enhanced immune responses can be carried out as is known in the art. For example, antibody titer can be measured by assays such as agglutination, immunoprecipitation, or ELISA.

Assays for chemotaxis relating to neutrophils are described in Walz *et al.* (1987), *Biochem. Biophys. Res. Commun. 149*: 755; Yoshimura et al. (1987), *Proc. Natl. Acad. Sci. USA 84*: 9233, and Schroder *et al.* (1987), *J. Immunol. 139:* 3474. Chemotaxis of lymphocytes can be assayed as described in Larsen *et al.*, *Science 243*: 1464: (1989) and Carr *et al.*, *Proc. Natl. Acad. Sci. USA 91:* 3652 (1994).

Assays for chemotaxis of tumor-infiltrating lymphocytes are described in Liao *et al.* (1995), *J. Exp. Med. 182*: 1301; for hemopoietic progenitors, in Aiuti *et al.* (1997), *J. Exp. Med. 185*: 111; for monocytes, in Valente *et al.* (1988), *Biochem. 27*:

4162; and for natural killer cells, in Loetscher *et al.* (1996), *J. Immunol. 156*: 322, and in Allavena *et al.* (1994), *Eur. J. Immunol. 24*: 3233.

Attraction or activation of eosinophils, dendritic cells, basophils, and neutrophils, can also be measured. Assays for determining eosinophil attraction are described in Dahinden *et al.*, *J. Exp. Med. 179*: 751 (1994), Weber *et al.*, *J. Immunol. 154*: 4166 (1995), and Noso *et al.*, *Biochem. Biophys. Res. Commun. 200*: 1470 (1994). Attraction of dendritic cells can be measured as described, for example, in Sozzani *et al.*, *J. Immunol.* 155: 3292 (1995). Assays for attracting basophils are taught in Dahinden *et al.*, *J. Exp. Med. 179*: 751 (1994), Alam *et al.*, *J. Immunol.* 152: 1298 (1994), and Alam *et al.*, *J. Exp. Med. 176*: 781 (1992). Activation of neutrophils is taught in Maghazaci *et al.*, *Eur. J. Immunol. 26*: 315 (1996) and Taub *et al.*, *J. Immunol. 155*: 3877 (1995). Cytotoxic T lymphocyte assays can also be used to measure enhanced immune response to a DNA immunogen (see Example 1, below).

The DNA immunogen can be any contiguous sequence of deoxyribonucleotides encoding a polypeptide which is capable of eliciting an immune response. For example, polynucleotides encoding immunogenic polypeptides of viruses such as HIV viruses (*e.g.*, gag, pol, or env), herpes viruses (*i.e.*, HSV-1, HSV-2), Epstein-Barr virus, varicella-zoster virus, cytomegalovirus, and hepatitis B virus (HBV), hepatitis C virus (HCV), and human papilloma viruses (*i.e.*, HPV-16, -18, and -31) can serve as a DNA immunogen. DNA which encodes polypeptide immunogens of other infectious agents, such as bacteria, fungi, or yeast, can function as a DNA immunogen in the method of the invention. DNA which encodes polypeptides specifically expressed by a tumor, such as EGFRvIII, Ras, or $p185^{HER2}$, or polypeptides which are expressed both by a tumor and by the corresponding normal tissue, can also function as a DNA immunogen. If desired, a DNA immunogen can comprise coding sequences for more than one immunogenic polypeptide..

A chemokine and a DNA immunogen can be administered to a mammal, preferably a human, by any means known in the art, including parenteral, intranasal, or intramuscular injection, or coated onto small metal projectiles and injected using a biological ballistic gun ("gene gun"). Alternatively, a chemokine and a DNA immunogen can be administered successively. The chemokine can be administered

prior to administration of the DNA immunogen, or the DNA immunogen can be administered prior to the administration of the chemokine.

A polynucleotide encoding the chemokine can also be administered. Preferably, a polynucleotide encoding the chemokine and a polynucleotide comprising the DNA immunogen are co-injected. The polynucleotides can also be administered successively, in any order. For co-administration, a single polynucleotide comprising both chemokine-encoding sequences and the DNA immunogen can be administered, or the DNA immunogen and the chemokine-encoding polynucleotide can be provided separately and mixed together prior to administration.

The invention also provides immunogenic compositions comprising a DNA immunogen and a chemokine or a polynucleotide encoding a chemokine. The composition can optionally comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules, such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates. Compositions of the invention can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes, such as those described in U.S. 5,422,120, WO 95/13796, WO 91/14445, or EP 524,968 B1, can also be used as a carrier for a composition of the invention.

Compositions of the invention can be used as vaccine compositions, for example, to enhance an immune response of a mammal, including a human, to an infectious agent or a tumor. The particular dosages of chemokine and DNA immunogen which are sufficient to enhance an immune response to the DNA immunogen will vary according to the chemokine and DNA immunogen being used and the mammal to which the chemokine and DNA immunogen are being administered. The amounts of each active agent in the examples described below provide general guidance for the range of each component to be utilized by the

practitioner upon optimizing the method of the present invention for practice either *in vitro* or *in vivo*. Generally, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, or 5 mg of a chemokine protein, a polynucleotide encoding a chemokine, or a polynucleotide comprising a DNA immunogen will be administered to a large mammal, such as a baboon or a human.

Such ranges by no means preclude use of a higher or lower amount of a component, as might be warranted in a particular application. For example, the actual dose and schedule may vary depending on whether the compositions are administered in combination with other pharmaceutical compositions or depending on individual differences in pharmacokinetics, drug disposition, and metabolism.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention described in broad terms above.

EXAMPLE 1

Co-administration of HCV immunogens and MIP-1 α increases lysis of autologous B cells infected with vaccinia virus encoding HCV polypeptide NS3

HCV immunogens. Each plasmid comprises a CMV enhancer/promoter and is Kanamycin-resistant. Plasmids were prepared by an alkaline lysis method from *E. coli* bacteria and purified using Qiagen purification systems. After purification, plasmids were stored at -80 °C, at a concentration of 1 mg/ml.

Plasmid pCMVKmΔNS comprises hepatitis C viral DNA encoding HCV polypeptides ΔNS3, NS4, NS5a, and NS5b (immunogen for animal Group 1). Plasmid NS-GM2 encodes HCV polypeptides ΔNS3, NS4, NS5b, NS5b, and hGM-CSF (immunogen for animal Group 2). Plasmid pCMVLhRantes encodes human RANTES protein. pCMVLhMIP1a encodes MIP-1α.

For the immunization protocols described below, pCMVKmΔNS was premixed with either pCMVLhRantes (immunogen for animal Group 3) or pCMVLhMIP1a (immunogen for animal Group 4). Each plasmid was at a concentration of 1 mg/ml of DNA, for a total of 2 mg/ml of DNA per mixed

immunogen.

Injection of HCV immunogens into baboons. On the day of injection, one vial (marked with the plasmid name and animal group) per animal was removed from the freezer, thawed at room temperature, and gently mixed. Each immunogen was injected both intramuscularly and intradermally. The total volume injected per animal was 1 ml.

The left and right tibialis anterior muscle was injected with 400 µl of DNA for a total of 800 µl intramuscular injection per baboon, using a 1 ml syringe. The immunogens were injected slowly, over about 10 seconds. After injection, the needle was removed slowly, to reduce leakage.

Each of two separates sites of the upper back was injected with 100 µl of DNA for a total of 200 µl intradermal injection per baboon, using a 0.3 ml U-100 Insulin syringe. The skin at the sites of injection was shaved. At each site, the needle was inserted the needle bevel up into the skin and then rotated 90 degrees so that the bevel pointed to the side. The 100 µl was slowly injected over about 10 seconds. After injection, the needle was slowly rotated so that the bevel was up again, then withdrawn slowly to reduce leakage.

Immunization and bleeding schedules for four groups of baboons. Baboons in each of four groups were immunized and bled according to the following schedule. Group 1 (animals CK544, CK545, CK546, and CK547) received inoculations of pCMVKmΔNS (HCV immunogens) and were bled according to the schedule in Figure 1. Group 2 (animals CK548, CK549, CK550, and CK551) received inoculations of NS-GM2 (HCV immunogens and GM-CSF) and were bled according to the schedule in Figure 2. Group 3 (animals CK552, CK553, CK554, and CK555) received inoculations of pCMVKmΔNS and pCMVLhRantes (HCV immunogens and RANTES) according to the schedule in Figure 3. Group 4 (animals CK556, CK557, CK558, and CK559) received inoculations of pCMVKmΔNS and pCMVLhMIP1a (HCV immunogens and MIP-1α) and were bled according to the schedule in Figure 4.

Immunizations were carried out as described in Example 2, above. At each of the times indicated in the bleeding schedules, blood was drawn from the femoral vein

while the baboons were under anesthesia (Ketamine®, 10 mg/ml). Blood was treated with heparin. B and T cells were isolated from these blood samples and used in the cytotoxic T lymphocyte assays described below.

CTL assays. Autologous B cell lines from each animal were established by transforming B cells with *H. papio*. Separate samples of peripheral blood mononuclear cells were restimulated with immortalized autologous B cells infected with a recombinant vaccinia virus that encodes each of the HCV immunogens (NS3, NS4, NS5a, and NS5b). Two weeks later, $CD8^+$ T lymphocytes were purified from the samples using magnetic beads.

The ability of T cells from each animal to lyse its autologous B cell line infected with vaccinia virus encoding the same immunogens used to immunize the animals was tested using a standard ^{51}Cr-release assay. Ratios of effector (T cells) to target (B cells) of 40:1, 10:1, and 2:1 were tested.

Percent lysis was calculated in each assay. A positive CTL response was noted if at least 10% more lysis occurred with homologous cells (stimulated with a vaccinia virus encoding an HCV immunogen) than with heterologous cells (stimulated with a vaccinia virus encoding an unrelated immunogen) for each of the two highest effector to target cell ratios tested.

Table I shows the number of animals with positive responses in a cytotoxic T lymphocyte assay.

Table I. Number of animals with CTL responses

Immunogen	No. of Animals
pCMVNS3-5	0/4
pCMVNS3-5 & MIP-1α	1/4
pCMVNS3-5 & RANTES	0/4

Table II shows percent lysis of target cells from animal CK556 after

restimulation. Homologous cells were stimulated with vaccinia virus encoding HCV polypeptide NS3.

Table II. Percent lysis of targets after restimulation (animal CK556)

	Effector:Target	Homologous[1]	Heterologous[2]
pre-immunization	40:1	2	11
pre-immunization	10:1	6	10
pre-immunization	2:1	7	6
2 weeks post 3rd immunization	40:1	27	<1
2 weeks post 3rd immunization	10:1	17	<1
2 weeks post 3rd immunization	2:1	11	<1

The results reported in Table II demonstrate that co-administration of HCV immunogens and the chemokine MIP-1α resulted in an increased lysis of autologous B cells infected with vaccinia virus encoding HCV polypeptide NS3.

EXAMPLE 2

Co-administration of HIV immunogens and BLC increases the titer of anti-p55gag

Balb/c mice received bilateral injections into the anterior tibialis muscle of 10 μg of a p55 plasmid, which encodes HIV gag, either alone or together with a total of 100 μg of a plasmid encoding B lymphocyte chemokine (BLC; *Nature 391*, 799-803, 1998). Fifty μg of BLC-encoding plasmid were injected into each muscle.

The animals were bled at 3 and 6 weeks after immunization, and anti-p55gag antibody titer was measured by ELISA. Figure 5 shows that anti-gag antibody titer in

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[1] stimulated with a vaccinia virus encoding HCV polypeptide NS3.

[2] stimulated with a vaccinia virus encoding an unrelated immunogen.

immunized mice is increased at three weeks after immunization and continues to increase up to at least six weeks.

NUMBERED REFERENCES

1. Ulmer, J., *et al.* 1993. Heterologous protection against influenze by injection of DNA encoding a viral protein [see comments]. *Science* 259:1745-9.

2. Fynan, E., R. *et al.* 1993. DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proc Natl Acad Sci USA* 90:11478-82.

3. Cox, G., *et al.* 1993. Bovine herpesvirus 1: immune responses in mice and cattle injected with plasmid DNA. *J Virol* 67:5664-7.

4. Sedegah, M., *et al.* 1994. Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein. *Proc Natl Acad Sci USA* 91:9866-70.

5. Barry, M., *et al.* 1995. Protection against mycoplasma infection using expression-library immunization. *Nature* 377-632-5.

6. Conry, R., *et al.* 1995. A carcinoembryonic antigen polynucleotide vaccine has in vivo antitumor activity. *Gene Ther* 2:59-65.

7. Syrengelas, A., *et al.*. 1996. DNA immunization induces protective immunity against B-cell lymphoma. *Nat Med* 2:1038-1041.

8. Tascon, R., *et al.* 1996. Vaccination against tuberculosis by DNA injection. *Nat Med* 2:888-92.

9. Yasutomi, Y., *et al.* 1996. Simian immunodeficiency virus-specific cytotoxic T-lymphocyte induction through DNA vaccination of rhesus monkeys. *J Virol* 70:678-81.

10. Letvin, M., *et al.* 1997. Potent protective anti-HIV immune responses generated by bimodal HIV envelope DNA plus protein vaccination. *Proc Natl Acad Sci USA* 94:9378-9383.

11. Xiang, Z., and H. Ertl. 1995. Manipulation of the immune responses to a plasmid-encoded viral antigen by coinoculation with plasmids expressing cytokines. *Immunity* 2:129-35.

12. Conry, R., *et al.* 1996. Selected strategies to augment polynucleotide immunization. *Gene Ther* 3:67-74.

13. Irvine, K., *et al.* 1996. Cytokine enhancement of DNA immunization leads to effective treatment of established pulmonary metastases. *J. Immunol* 156:238-45.

14. Chow, Y., *et al.* 1997. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B Surface antigen and interleukin-2. *J Virol* 71:169-78.

15. Iwasaki, A., *et al.* 1997. Enhanced CTL responses mediated by plasmid DNA immunogens encoding costimulatory molecules and cytokines. *J Immmunol* 158:4591-601.

16. Kim, J., *et al.* 1997. In vivo engineering of a cellular immune response by coadministration of IL-12 expression vector with a DNA immunogen. *J Immunol* 158:816-26.

17. Okada, E., *et al.* 1997. Intranasal immunization of a DNA vaccine with IL-12- and granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens. *J Immunol* 159:3638-47.

18. Geissler, M., *et al.* 1997. Enhancement of cellular and humoral immune responses to hepatitis C viruws core proteins using DNA-based vaccines augmented with cytokine-expressing plasmids. *J Immunol* 158:1231-7.

19. Larsen, D., *et al.* 1998. Coadministration of DNA encoding interleukin-6 and hemagglutinin confers protection from influenza virus challenge in mice. *J Virol* 72:1704-8.

20. Butcher, E. 1991. Leukocyte-endothelial cell recogtnition: three (or more) steps to specificity and diversity. *Cell* 67:1033-6.

21. Springer, T. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76:301-14.

22. Butcher, E., and L. Picker. 1996. Lymphocyte homing and homeostasis. *Science* 272:60-6.

23. Schall, T., and K. Bacon. 1994. Chemokines, leukocyte trafficking, and inflammation. *Curr Opin Immunol* 6:865-73.

24. Taub, D., *et al.* 1993. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 alpha and MIP-1 beta *Science* 260:355-8.

25. Schall, T., *et al.* 1993. Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. *J Exp Med* 177:1821-6.

26. Schall, T., *et al.* 1990. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 347:669-71.

27. Rot, A., *et al.* 1992. RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 176:1489-95.

We claim:

1. An immunogenic composition, comprising:

a DNA immunogen; and

a chemokine or a polynucleotide encoding a chemokine.

2. The immunogenic composition of claim 1 wherein the DNA immunogen comprises a polynucleotide encoding a viral immunogen.

3. The immunogenic composition of claim 2 wherein the polynucleotide encodes a hepatitis C virus non-structural polypeptide.

4. The immunogenic composition of claim 3 wherein the hepatitis C virus non-structural polypeptide is selected from the group consisting of NS3, NS4, NS5a, and NS5b.

5. The immunogenic composition of claim 2 wherein the polynucleotide encodes an HIV polypeptide.

6. The immunogenic composition of claim 5 wherein the HIV polypeptide is a gag polypeptide.

7. The immunogenic composition of claim 1 wherein the DNA immunogen comprises a polynucleotide encoding an immunogen expressed by a tumor.

8. The immunogenic composition of claim 1 wherein the chemokine is macrophage inflammatory protein 1α (MIP-1α).

9. The immunogenic composition of claim 1 wherein the chemokine is B lymphocyte chemokine (BLC).

10. The immunogenic composition of claim 1 further comprising a pharmaceutically acceptable carrier.

11. A method of enhancing an immune response to a DNA immunogen in a mammal, comprising the step of:

administering to the mammal (i) a chemokine or a first polynucleotide encoding a chemokine and (ii) a DNA immunogen, whereby an immune response to the DNA immunogen is enhanced.

12. The method of claim 11 wherein a chemokine is administered.

13. The method of claim 12 wherein the chemokine and the DNA immunogen are co-administered.

14. The method of claim 12 wherein the chemokine is administered prior to administration of the DNA immunogen.

15. The method of claim 12 wherein the DNA immunogen is administered prior to administration of the chemokine.

16. The method of claim 11 wherein a first polynucleotide encoding the chemokine is administered.

17. The method of claim 16 wherein the first polynucleotide and the DNA immunogen are co-administered.

18. The method of claim 16 wherein the polynucleotide is administered prior to administration of the DNA immunogen.

19. The method of claim 16 wherein the DNA immunogen is administered prior to administration of the first polynucleotide.

20. The method of claim 16 wherein a second polynucleotide which comprises(a) the first polynucleotide and (b) the DNA immunogen is administered.

21. The method of claim 11 wherein the chemokine is macrophage inflammatory protein 1α (MIP-1α).

22. The method of claim 11 wherein the chemokine is B lymphocyte chemokine (BLC).

23. The method of claim 11 wherein the DNA immunogen comprises a polynucleotide which encodes a hepatitis C virus non-structural polypeptide.

24. The method of claim 23 wherein the hepatitis C virus non-structural polypeptide is selected from the group consisting of NS3, NS4, NS5a, and NS5b.

25. The method of claim 23 wherein the polynucleotide encodes an HIV polypeptide.

26. The method of claim 25 wherein the HIV polypeptide is a gag polypeptide.

27. The method of claim 11 wherein the mammal is a human.

28. The method of claim 11 wherein the immune response is an antibody response.

29. The method of claim 11 wherein the immune response is a cytotoxic T lymphocyte response.

CHIRON #	SFBR#	SEX	Wt. kg
CX544	11117		
CX545	11281		
CX546	10832		
CX547	11272		
		AVERAGE=	#DIV/01

FIG. 1

STATUS	DATE	TOTAL TIME(WKS)	IMM#	IMMUNOGEN	BLEEDING SCHEDULE BLEED#	BLEEDING SCHEDULE CYCLE "TIME"	CHIRON CLOTTED BLOOD FOR SERUM (ml)	CHIRON HEPA-RINIZED BLOOD BY (ml)	???? OTHER	TOTAL PER TIME (ml)	% OF MAX BLEED VOL	PERIOD
	NA		–	–		–	0			0		13
	NA		–	–		–	0	0		0		0
	NA		–	–		–	0	0		0		0
NEW	9/23/97	-2.0	–	–	prebld 0	–	0	20		* 20		1
	10/7/97	0.0	1	pCMVKmANS		0.0	0	0		0		1
	11/4/97	4.0	2	pCMVKmANS		4.0	0	0		0		1
	11/18/97	6.0			1	8.0	0	20		* 20		1
	12/18/97	10.0			2	10.0	0	20		* 20	#DIV/01	1
	1/8/98	13.0	3	pCMVKmANS		#REFI	0	0		0		2
	1/20/98	16.0			3	#REFI	0	20		* 20		2
	2/17/98	19.0			4	#REFI	0	20		* 20		2
	3/17/98	23.0			5	#REFI	0	20		* 20	#DIV/01	2
	4/7/98	26.0	4	pCMVKmANS		#REFI	0	0		0		3
	4/21/98	28.0			6	#REFI	0	20		* 20		3
	5/19/98	32.0			7	#REFI	0	20		* 20		3
	6/16/98	36.0			8	#REFI	0	20		* 20	#DIV/01	3
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999

CHIRON #	SFBR#	SEX	Wt. kg
CX548	11538		
CX549	8854		
CX550	9421		
CX551	10645		
		AVERAGE=	#DIV/01

FIG. 2

STATUS	DATE	TOTAL TIME(WKS)	IMM#	IMMUNOGEN	BLEEDING SCHEDULE BLEED#	BLEEDING SCHEDULE "TIME"	CHIRON CLOTTED BLOOD FOR SERUM (ml)	CHIRON HEPA-RINIZED BLOOD BY (ml)	???? OTHER	TOTAL PER TIME (ml)	% OF MAX BLEED VOL	PERIOD 13
	NA		-	-		-	0			0		0
	NA		-	-		-	0	0		0		0
	NA		-	-		-	0	0		0	#DIV/01	0
NEW	9/25/97	-2.0	-	-	prebld 0	-	0	20		* 20		1
	10/9/97	0.0	1	NS-GM2		0.0	0	0		0		1
	11/6/97	4.0	2	NS-GM2		4.0	0	0		0		1
	11/20/97	6.0			1	8.0	0	20		* 20		1
	12/18/97	10.0			2	10.0	0	20		* 20	#DIV/01	1
	1/8/98	13.0	3	NS-GM2		#REFI	0	0		0		2
	1/22/98	16.0			3	#REFI	0	20		* 20		2
	2/19/98	19.0			4	#REFI	0	20		* 20		2
	3/19/98	23.0			5	#REFI	0	20		* 20	#DIV/01	2
	4/9/98	26.0	4	NS-GM2		#REFI	0	0		0		3
	4/23/98	28.0			6	#REFI	0	20		* 20		3
	5/21/98	32.0			7	#REFI	0	20		* 20		3
	6/18/98	38.0			8	#REFI	0	20		* 20	#DIV/01	3
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999

SUBSTITUTE SHEET (RULE 26)

CHIRON #	SFBR#	SEX	Wt. kg
CX552	9047		
CX553	9801		
CX554	10593		
CX555	10399		
		AVERAGE=	#DIV/01

FIG. 3

STATUS	DATE	TOTAL TIME(WKS)	IMM#	IMMUNOGEN	BLEEDING SCHEDULE BLEED#	BLEEDING SCHEDULE CYCLE "TIME"	CHIRON CLOTTED BLOOD FOR SERUM (ml)	CHIRON HEPA-RINIZED BLOOD BY (ml)	???? OTHER	TOTAL PER TIME (ml)	% OF MAX BLEED VOL	PERIOD 13
	NA		–	–		–	0			0		0
	NA		–	–		–	0	0		0		0
	NA		–	–		–	0	0		0	#DIV/01	0
NEW	10/7/97	-2.0	–	–	prebld 0	–	0	20		* 20		1
	10/21/97	0.0	1	pCMVKmANS+pCMVLhRanise		0.0	0	0		0		1
	11/18/97	4.0	2	pCMVKmANS+pCMVLhRanise		4.0	0	0		0		1
	12/2/97	6.0			1	8.0	0	20		* 20		1
	12/30/97	10.0			2	10.0	0	20		* 20	#DIV/01	1
	1/20/98	13.0	3	pCMVKmANS+pCMVLhRanise		#REFI	0	0		0		2
	2/3/98	16.0			3	#REFI	0	20		* 20		2
	3/3/98	19.0			4	#REFI	0	20		* 20		2
	3/31/98	23.0			5	#REFI	0	20		* 20	#DIV/01	2
	4/21/98	26.0	4	pCMVKmANS+pCMVLhRanise		#REFI	0	0		0		3
	5/5/98	28.0			6	#REFI	0	20		* 20		3
	6/2/98	32.0			7	#REFI	0	20		* 20		3
	6/30/98	36.0			8	#REFI	0	20		* 20	#DIV/01	3
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999

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CHIRON #	SFBR#	SEX	Wt. kg
CX556	10850		
CX557	9009		
CX558	7750		
CX559	10242		
		AVERAGE=	#DIV/01

FIG. 4

STATUS	DATE	TOTAL TIME(WKS)	IMM#	IMMUNOGEN	BLEEDING SCHEDULE BLEED#	BLEEDING SCHEDULE CYCLE "TIME"	CHIRON CLOTTED BLOOD FOR SERUM (ml)	CHIRON HEPA-RINIZED BLOOD BY (ml)	???? OTHER	TOTAL PER TIME (ml)	% OF MAX BLEED VOL	PERIOD 13
	NA		–	–		–	0			0		0
	NA		–	–		–	0	0		0		0
	NA		–	–		–	0	0		0	#DIV/01	0
NEW	10/9/97	-2.0	–	–	prebld 0	–	0	20		* 20		1
	10/23/97	0.0	1	pCMVkmANS+pCMVLhMIP1a		0.0	0	0		0		1
	11/20/97	4.0	2	pCMVkmANS+pCMVLhMIP1a		4.0	0	0		0		1
	12/4/97	6.0			1	6.0	0	20		* 20		1
	1/1/98	10.0			2	10.0	0	20		* 20	#DIV/01	1
	1/22/98	13.0	3	pCMVkmANS+pCMVLhMIP1a		#REFI	0	0		0		2
	2/5/98	16.0			3	#REFI	0	20		* 20		2
	3/5/98	19.0			4	#REFI	0	20		* 20		2
	4/2/98	23.0			5	#REFI	0	20		* 20	#DIV/01	2
	4/23/98	26.0	4	pCMVkmANS+pCMVLhMIP1a		#REFI	0	0		0		3
	5/7/98	28.0			6	#REFI	0	20		* 20		3
	6/4/98	32.0			7	#REFI	0	20		* 20		3
	7/2/98	38.0			8	#REFI	0	20		* 20	#DIV/01	3
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999
	TBD	TBD					0			0		999

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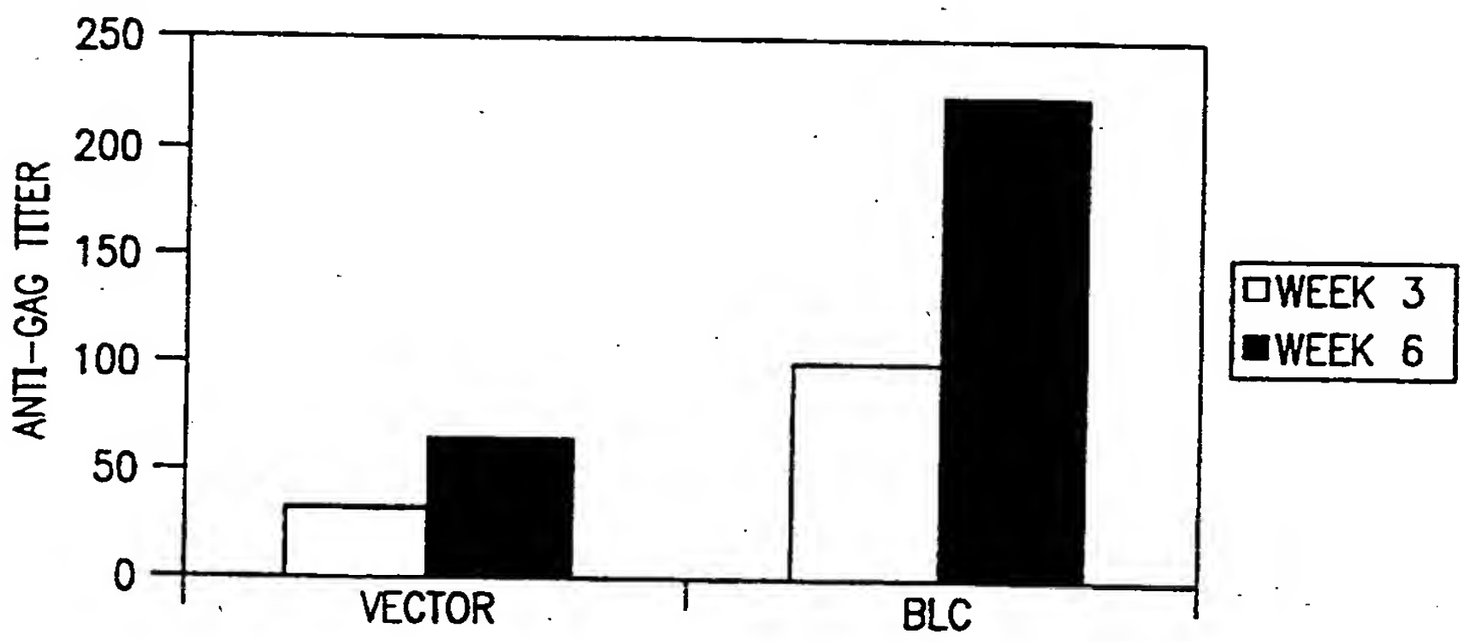
250
200
150
100
50
0
ANTI-GAG TITER
VECTOR
BLC
PLASMID CO-INJECTED
WEEK 3
WEEK 6

FIG. 5

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/36, 15/49, A61K 48/00

A3

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(71) Applicant *(for all designated States except US)*: CHIRON CORPORATION [US/US]; Intellectual Property – R440, P.O. Box 8097, Emeryville, CA 94622–8097 (US).

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(74) Agents: HARBIN, Alisa, A. et al.; Chiron Corporation, Intellectual Property – R440, P.O. Box 8097, Emeryville, CA 49662–8097 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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23 December 1999 (23.12.99)

(54) Title: ENHANCING IMMUNE RESPONSES TO GENETIC IMMUNIZATION BY USING A CHEMOKINE

(57) Abstract

The immune response to a DNA immunogen in a mammal can be enhanced by administration of a chemokine or a polynucleotide encoding the chemokine. This method can be used, for example, to immunize or vaccinate a mammal against an infectious disease or a tumor.

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INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 99/08802

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/36 C12N15/49 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 96 36366 A (DOW STEVE W ;ELMSLIE ROBYN E (US); NAT JEWISH CENTER FOR IMMUNOLO) 21 November 1996 (1996-11-21) page 6, line 19 -page 8, line 18 page 17, line 24 -page 20, line 3 page 53, line 4 -page 56, line 23 page 89, line 5 -page 92, line 15 ---	1,2,7,8, 10-21, 27-29 3-6, 23-26
X Y	WO 94 28916 A (BRITISH BIOTECH PHARM ;COMER MICHAEL BERISFORD (GB); MCCOURT MATTH) 22 December 1994 (1994-12-22) page 4, line 31 -page 5, line 11 page 14, line 24 -page 17, line 27 page 25, line 20 -page 26, line 6 ---	1,2,5-8, 10-21, 25-29 3,4,23, 24

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 October 1999	03/11/1999
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Sitch, W

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/08802

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 15285 A (WISTAR INST ;ERTL HILDEGUND C J (US); THURIN MAGDALENA (US)) 16 April 1998 (1998-04-16) page 4, line 24 -page 6, line 28 ---	1,7, 10-20, 27-29
X	WO 96 11279 A (US HEALTH) 18 April 1996 (1996-04-18) page 18, line 20 -page 21, line 29 page 24, line 29 -page 28, line 7 ---	1,2,5-7, 10-21, 25-29
Y	KHUDYAKOV Y E ET AL: "LINEAR B-CELL EPITOPES OF THE NS3-NS4-NS5 PROTEINS OF THE HEPATITISC VIRUS AS MODELED WITH SYNTHETIC PEPTIDES" VIROLOGY, vol. 206, 1 January 1995 (1995-01-01), pages 666-672, XP000574456 ISSN: 0042-6822 page 666 abstract ---	3,4,23, 24
Y	MOLDOVEANU Z ET AL: "IMMUNE RESPONSES INDUCED BY ADMINISTRATION OF ENCAPSIDATED POLIOVIRUS REPLICONS WHICH EXPRESS HIV-1 GAG AND ENVELOPE PROTEINS" VACCINE, vol. 13, no. 11, 1 August 1995 (1995-08-01), pages 1013-1022, XP000571592 ISSN: 0264-410X page 1013 abstract ---	5,6,25, 26
A	WO 97 19696 A (LUSSO PAOLO ;GALLO ROBERT C (US); COCCHI FIORENZA (US); VICO ANTHO) 5 June 1997 (1997-06-05) page 4, line 11 -page 5, line 20 ---	
A	DILLOO ET AL: "COMBINED CHEMOKINE AND CYTOKINE GENE TRANSFER ENHANCES ANTITUMOR IMMUNITY" NATURE MEDICINE, vol. 2, no. 10, October 1996 (1996-10), pages 1090-1095, XP002119425 page 1090 abstract ---	
E	WO 99 29728 A (GALLO ROBERT C ;DEVICO ANTHONY L (US); GARZINO DEMO ALFREDO (US);) 17 June 1999 (1999-06-17) page 12, paragraph 3 -page 25, paragraph 2 -----	1,2,5,6, 8-22, 25-29

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 08802

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 11-29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTER''ATIONAL SEARCH REPORT

Information on patent family members

ıational Application No
PCT/US 99/08802

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9636366 A	21-11-1996	US 5705151 A	06-01-1998
		US 5935568 A	10-08-1999
		AU 704012 B	01-04-1999
		AU 5801696 A	29-11-1996
		CA 2221305 A	21-11-1996
		EP 0850071 A	01-07-1998
		JP 11508762 T	03-08-1999
WO 9428916 A	22-12-1994	AU 6974294 A	03-01-1995
		EP 0703784 A	03-04-1996
		JP 8511263 T	26-11-1996
		US 5925568 A	20-07-1999
		ZA 9404258 A	15-12-1995
WO 9815285 A	16-04-1998	AU 4907797 A	05-05-1998
WO 9611279 A	18-04-1996	AU 3998295 A	02-05-1996
		CA 2201592 A	18-04-1996
		EP 0789774 A	20-08-1997
WO 9719696 A	05-06-1997	AU 1141997 A	19-06-1997
		EP 0869812 A	14-10-1998
WO 9929728 A	17-06-1999	AU 1815899 A	28-06-1999